that were more satisfying than we had originally hoped for.

What has changed most in the field since you began to work in it? Well, there was no molecular biology going on when we showed up in the early 1980s. It was a time when people were just starting to isolate genes in just a few model organisms, and our target in *Drosophila* happened to control this behavior. The first meeting I attended in this field was a Gordon Conference on Chronobiology. For the molecular talks you had me, Jeff Hall and Michael Rosbash just giving a first peak at *per*. There were developing genetic studies in *Neurospora* that very quickly blossomed in the next few years, but had not gone molecular yet. Fascinating transplantation studies were giving anatomical localizations for neural pacemakers. There were also intriguing electrophysiological studies and some suggestive biochemical experiments looking at time-of-day specific inhibitors of RNA and protein synthesis that affected circadian rhythmicity. Those were being used to argue that unknown proteins controlling the clock were present at only some times of day, which of course turned out to be true and an important prediction.

It seems everyone who was interested in biological clocks then has now become very good at genetics and molecular biology. The range of systems currently understood in depth is remarkable. There has been a profound level of tool development and analysis that can now focus on everything from monitoring multiple gene rhythms in live cells in culture to studies of complex rhythms in clusters of neurons in behaving mice. We've become one of the big beneficiaries of all of this activity: our work is still centered on *Drosophila*, but there are also new projects that we would not have approached a few years ago. For instance, we're using primary skin cultures to look directly at human circadian biology. It's an unusual community that has remained very open and collaborative. We get an encouraging push from time to time to take on new ventures.

Laboratory of Genetics, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.
E-mail: young@rockefeller.edu

Quick guide

Mauthner cells

Keith T. Sillar

What are Mauthner cells and why are they interesting? Tapping an aquarium tank and watching the fish dart away re-enacts, perhaps unwittingly, a simple neuroethological experiment. This startle response rapidly causes the fish's body to adopt a characteristic C-shape (Figure 1, inset), enabling it to orient away from danger before swimming off at high speed. At the centre of the neural pathway underlying the C-start response are two reticulospinal neurons, called Mauthner cells, located in the hindbrain near the entry of cranial nerve VIII, just beneath the fourth ventricle. They are identified by their relatively huge somata, crescent shaped dendrites and large diameter, myelinated axons that cross over and project caudally down the contralateral spinal cord (Figure 1). Their large size and accessibility render them a valuable model in which to study basic mechanisms in cellular neurobiology and the neuronal basis of behaviour. Indeed Mauthner cell research has greatly enhanced understanding of numerous general principles in neuroscience, such as the command neuron concept, electrical transmission, quantal transmitter release and synaptic plasticity. Few neurons have an entire book dedicated to them!

How are Mauthner cells activated? The main sensory inputs that trigger firing of Mauthner cells are from ipsilateral auditory hair cells (Figure 1), but other afferents of the vestibular and lateral line system also make direct, normally sub-threshold excitatory connections. The auditory synapses are specialized points of contact called club endings located on the lateral dendrite of the Mauthner cell, where both chemical and electrical transmission takes place. The presence of gap junctions provides a fast electrical component to the postsynaptic response with a minuscule delay of about 0.1 milliseconds, ensuring the rapid activation of the Mauthner cell following VIIIth nerve stimulation.

Which neurons do Mauthner cells activate? A cascade of events inevitably follows Mauthner cell activation (Figure 1). Firstly, the action potential propagates at high velocity along the axon in the contralateral spinal cord. Secondly, the opposing Mauthner cell is inhibited by both a conventional chemical inhibitory post-synaptic potential (IPSP) and a rare form of electrical inhibition, thereby ensuring the two never fire together. In the spinal cord, the Mauthner cell makes a multitude of synaptic connections, particularly with the large primary motorneurons innervating the contralateral trunk and tail muscles. The high conduction velocity of the Mauthner cell ensures these motorneurons discharge almost synchronously along the length of the body. In a 10 centimetre goldfish, a Mauthner cell axon conducting at 100 metres per second would take only 1 millisecond to propagate through the entire spinal cord. The resulting contraction bends the body into a characteristic C-shape with the head pointing away from stimulus (Figure 1, inset). Mauthner cells also excite spinal commissural inhibitory interneurons, ensuring the ipsilateral muscles cannot contract. Finally, Mauthner cells couple to excitatory premotor interneurons involved in generating swimming. This serial and parallel activation of escape and non-escape circuitry coordinates an appropriate temporal sequencing of escape.

What role do Mauthner cells play in behaviour? Mauthner cells can be viewed as 'command' neurons for escape, but what evidence supports this view? If the strict criteria of sufficiency and necessity are applied, it could be argued they are not true command neurons because C-starts occur even when the Mauthner cell is ablated and artificial activation of a Mauthner cell can fail to produce normal escape. However, *in vivo* imaging and ablation studies in zebrafish larvae have shown that the Mauthner cell is indeed activated during startle escape behaviour. When the Mauthner cell is ablated, C-starts can still be evoked because other reticulospinal cells are

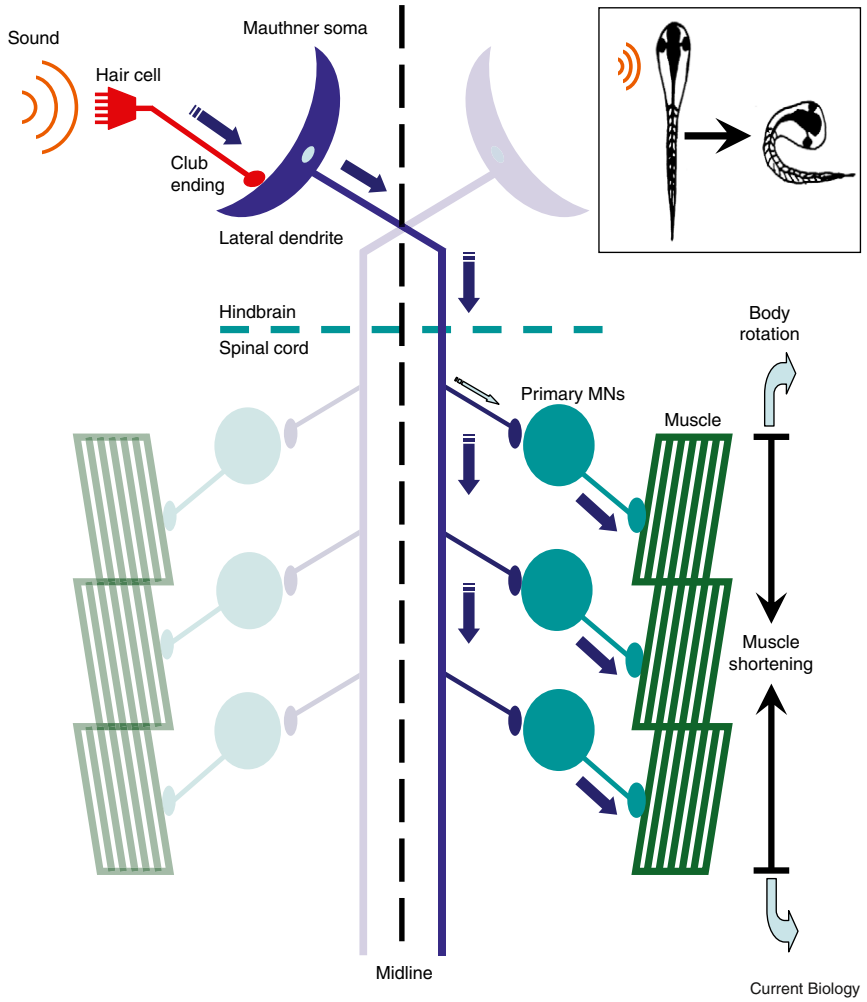

Figure 1. Schematic representation of the Mauthner cell circuitry of the goldfish.
A simulated response to sound on the left is shown with blue arrows indicating the progress of activity from hair cell afferent to contralateral primary motorneurons (primary MNs) and muscles on the right via the Mauthner cell. Equivalent Mauthner circuitry on the other side is shown shaded.

activated in parallel. Because of their smaller axons, however, these Mauthner homologues elicit delayed C-starts. In the race to escape, delays of even milliseconds might result in being someone's lunch, so command neurons like the Mauthner cell increase the chances of survival.

Can't predators predict the direction of escape? The main function of the Mauthner cell-mediated escape reflex is to evade predation, but if the response always took the same direction a predator could quickly anticipate where to strike. Fortunately, C-starts are inherently flexible so the final response trajectory has in-built variability; sometimes fish execute a second C-start shortly after the first, turning them roughly towards the unsuspecting predator! The escape behaviour is recognized as having two distinct phases: an initial, relatively stereotyped phase (the C-start) and a second later, more variable phase (beginning escape swimming), which determines the final orientation of the fish.

This unpredictability of response trajectory may be a general anti-predatory feature to emerge from escape circuits. For example, a rather similar strategy has been adopted by the cockroach (see Domenici *et al.* (2008). Cockroaches keep predators guessing by using preferred escape trajectories. Curr. Biol. *18*, 1792-1796). In this case, however, the animal escapes from threats detected by wind-sensitive cercal afferents by running away at high speed along one of a set of preferred, distinct trajectories which predators presumably cannot predict. The common theme though is that built in variability keeps predators guessing.

What affects the decision to escape? C-start escape behaviour is a highly energetic and attention-grabbing manoeuvre, so the decision to escape cannot be taken lightly. The Mauthner cell threshold is therefore set high to prevent innocuous inputs from triggering escape. The activation threshold is not fixed, however, because auditory club ending synapses display profound synaptic plasticity, being subject to short and long-term potentiation and depression under appropriate experimental conditions. Neuromodulation may underlie this plasticity. For example, both the chemical and electrical components of the Mauthner cell response are potentiated by endocannabinoids. But what is the point of building such flexibility into the design of an escape system? Perhaps, in a constantly changing acoustic environment, synaptic plasticity and neuromodulation are important for behavioural adaptations, such as habituation and sensitization. The Mauthner cell circuit provides potentially fruitful research avenues for future studies of how neuromodulators shape the output of neural networks underlying behaviour.

What about the evolution of Mauthner cells? There are strong selection pressures on the evolution of neural circuits that enable rapid escape because, in the immortal words of Joe Fetcho (1991):

"Being eaten alive abruptly ends all chances of future reproduction and is not favourable from an evolutionary view point"

From a phylogenetic perspective, Mauthner cells are identifiable not only in fish but also in amphibians, where they are particularly conspicuous in tadpoles, which swim like fish. The Mauthner cell system, in common with escape circuits in other phyla, incorporates special design features that enhance the speed of escape, including relatively few neurons in the pathway, large diameter, fast conducting axons and electrical synapses to speed transmission. Perhaps because of its command

function in executing fast escape, however, the Mauthner system has been evolutionarily malleable, having been incorporated into a range of modified C-start behaviours. For example, goldfish use C-starts during prey capture as well as predator avoidance. Similarly, archer fish have evolved a dramatic prey capture mechanism, whereby the retrieval of insects dislodged from vegetation by a spit of water involves a C-start with the hallmarks of Mauthner cell involvement. Recent evidence suggests that flying fish become airborne using an adapted system in which Mauthner cells connect to fin adductor motorneurons. In contrast to C-starts, however, left and right fin motorneurons are activated simultaneously, producing a sufficiently powerful bilateral fin adduction for an aerial escape. A similar adaptation occurs in the Mauthner cell system of anuran amphibians. Whilst in larval stages the Mauthner cells mediate classical C-starts, the cells atrophy as the tail regresses during metamorphosis but are retained in limbed juveniles to mediate a powerful, synchronous contraction of the two hind legs in a diving startle response which propels them away from danger.

In conclusion, Mauthner cells have evolved to maximize the speed of escape and hence optimize survival. During evolution, the Mauthner system has become incorporated into modified escape and predatory behaviours suiting the morphological, behavioural and ecological constraints of the host organism.

Where can I find out more?

Cachope, R., Mackie, K., Triller, A., O'Brien, J., and Pereda, A. (2007). Potentiation of electrical and chemical synaptic transmission mediated by endocannabinoids. Neuron *56*, 1034–1037.

Eaton, R.C., Lee, R.K.K., and Foreman, M.B. (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. Prog. Neurobiol. *63*, 467–485.

Faber, D.S., and Korn, H. (1978). Neurobiology of the Mauthner Neuron. Raven Press.

Fetcho, J. (1991). The spinal network of the Mauthner cell. Brain Behav. Evol. *37*, 298–316.

Korn, H., and Faber, D.S. (2005). The Mauthner cell half a century later: a neurobiological model for decision making. Neuron *47*, 13–28.

Liu, K.S., and Fetcho, J.R. (1999). Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish. Neuron *23*, 325–335.

School of Biology, University of St Andrews, St Andrews, Fife KY16 9TS, Scotland, UK.
E-mail: kts1@st-andrews.ac.uk

Primer

Orchestration of the immune response by dendritic cells

Matthew R. Buckwalter and Matthew L. Albert*

The immune system is arguably one of the most complex cellular organizations that exists in the body. This system is composed of multiple cell types that are arranged in distinct organs or circulate through the blood and peripheral tissues. The complexity of the immune system is not superfluous, but rather it is required to fulfill the multifaceted purpose of the immune system, namely: the recognition of the diverse repertoire of micro-organisms; the detection of neoplastic legions originating from a range of tissues; and, while executing these tasks, the maintenance of peripheral tolerance by suppressing detrimental responses against healthy tissues. Dendritic cells are critical players in conducting the immune response to fulfill these roles. Here we provide an overview of how dendritic cells monitor their surrounding environment and coordinate an appropriate response during both steady-state and inflammatory conditions. We also highlight some of the current approaches aimed to harness the unique properties of these cells for use as therapeutic agents against cancer and infectious disease.

Discovery

The term 'dendritic' was first used by Ralph Steinman and Zanvil Cohn in 1973 to describe a novel cell type identified in the secondary lymphoid organs of mice. Using microscopy techniques, they characterised this relatively rare population (~1%) on the basis of its adherence properties and morphology, with the most striking feature being its long cytoplasmic processes, which extend and retract from the cell body. A physiological role for this newly discovered cell type was not immediately appreciated. It was several years before dendritic cells were identified as 'accessory cells', which demonstrated a capacity, greater than that of macrophages, to stimulate allogeneic lymphocytes. Another important milestone in the understanding of dendritic cell biology was the discovery that these cells have the ability to present antigen on major histocompatibility complex (MHC) class I and II molecules. Upon migration and maturation, dendritic cells become capable of engaging lymphocytes and initiating immune programs. These observations led to dendritic cells being regarded as the 'sentinels' of the immune system and also as 'professional' antigen-presenting cells, for their multiple roles in orchestrating immunity.

Dendritic cell subsets

Dendritic cells have been categorized into multiple subsets with the two broadest categories being conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). cDCs can be further subdivided into distinct populations on the basis of their origin, location and differential expression of surface markers. For example, multiple subtypes of cDC exist in the skin: the cDCs in the epidermis are termed Langerhans cells and possess unique structures called Birbeck granules; both Langerhans cells and dermal dendritic cells (also called interstitial dendritic cells) are present in the dermal layer; and, during inflammatory processes, there is infiltration by monocytes that may differentiate into a third subset of cDCs termed monocyte-derived cDCs. Tissue cDCs, such as the three mentioned, are typically referred to as 'immature', on the basis of their ability to capture antigen and their modest capacity to stimulate T cells. Upon activation, these 'immature' cells may differentiate and migrate via the afferent lymphatics into draining lymph nodes. Upon maturation, cDCs downregulate their ability to capture antigen and now possess an enhanced ability to stimulate T cells. Secondary lymphoid tissues, such as lymph nodes and spleen, therefore contain migratory tissue cDCs that have been stimulated to 'mature', but they also contain resident populations of cDCs that have the ability to capture and process internalized antigen. Unique functions have been ascribed to distinct populations of cDCs; however, the overlapping roles and diversity of responses are more complex than a simple